

The Haemolytic Effects of Diaphenylsulfone (DDS) in Normal Subjects and in those with Glucose-6-phosphate-dehydrogenase Deficiency*

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The need to investigate further the phenomenon of sulfone-induced haemolysis is becoming greater as the use of sulfones may increase, particularly for malaria therapy in areas where Plasmodium falciparum is found to be resistant to chloroquine. The authors report on studies of the haemolytic effects of diaphenylsulfone (DDS) administered orally, in doses ranging from 25 mg to 300 mg daily for 21 days, to normal healthy men and to healthy Negro men with deficiency of glucose-6-phosphate dehydrogenase (G-6-PD). The latter proved more susceptible to diaphenylsulfone-induced haemolysis than did normal men. There was a direct relationship between the dose of diaphenylsulfone and the extent of haemolysis in both groups of men studied. Comparison of the haemolytic effects of diaphenylsulfone with those of the antimalarial drug primaquine revealed that, on a dose for weight basis, diaphenylsulfone is more haemolytic than primaquine in normal persons and less so in G-6-PD-deficient persons. A marked decrease in the content of reduced glutathione (GSH) in red cells, comparable to the changes in levels of erythrocytic GSH known to occur during primaquine-induced haemolysis, occurred just before and early during the acute haemolytic episode that resulted from administration of diaphenylsulfone to G-6-PD-deficient subjects; in contrast, levels of erythrocytic GSH increased early during the course of diaphenylsulfone-induced haemolysis in normal men.

Haemolysis constitutes one of the chief undesirable side-effects attending the administration of sulfones. Although sulfones have been employed extensively, particularly in the treatment of leprosy (Bushby, 1964), many aspects of sulfone-induced haemolysis remain unsettled. This report describes the results of detailed studies on the haemolytic effects of diaphenylsulfone³ (4,4'-diaminodiphenylsulfone;

DDS) administered to normal Caucasian or American Negro men and to American Negro men deficient in glucose-6-phosphate dehydrogenase (G-6-PD).

METHODS

Experimental design and objectives

Diaphenylsulfone was administered in doses ranging from 25 mg to 300 mg daily to 15 volunteers (10 normal men and five Negro men who were G-6-PD-deficient). The planned duration of treatment was 21 days. Four other volunteers (three normal men and one G-6-PD-deficient Negro man), who received no diaphenylsulfone, served as "control" subjects. Studies were performed chiefly (1) to obtain information about relationships between the dose of diaphenylsulfone and the extent of haemolysis in normal and in G-6-PD-deficient individuals; (2) to obtain data concerning biochemical changes associated with diaphenylsulfone-induced haemo-

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lysis, particularly those involving erythrocytic glutathione; and (3) to compare the haemolytic effects of diaphenylsulfone with those of primaquine.

Volunteers

The 19 volunteers who participated in these studies were inmates of the Illinois State Penitentiary, Stateville Branch, Joliet, Ill., USA. All volunteers were in excellent health as judged by a careful review of each volunteer's medical history, physical examination, chest X-ray, blood tests (levels of the haematocrit and of haemoglobin, leukocyte counts, and differential leukocyte counts), urinalysis, and phenolsulfonphthalein test. The presence or absence of G-6-PD deficiency was determined with the methaemoglobin-reduction test of Brewer and co-workers (1962); findings were confirmed by assays of the activity of G-6-PD in haemolysates measured by the method of Glock & McLean (1953) with modifications similar to those of Zinkham & Lenhard (1959). Several different genetic variants of G-6-PD deficiency have been described; studies concerning G-6-PD-deficient individuals presented in this report pertain only to American Negro men with this deficiency.

Volunteers were hospitalized throughout each study. From each subject 15 ml of venous blood, anticoagulated with heparin, were withdrawn at 6.30 a.m. (before breakfast) each day for all laboratory studies performed that day. Studies were carried out for eight days before administration of diaphenylsulfone (to obtain "baseline" information), during ingestion of diaphenylsulfone, and for eight days thereafter. Volunteers treated with diaphenylsulfone received single oral doses administered at 6.45 a.m. Administration of the drug was terminated before completion of the planned 21-day course of medication during studies with two of four normal subjects who received 300 mg of diaphenylsulfone daily (Volunteers 2 and 3). Volunteer 2 became agitated and nervous, and ingestion of the drug was discontinued, on the twentieth day of treatment; his symptoms subsided the next day. Volunteer 3 received 300 mg of diaphenylsulfone daily for 12 days followed by 200 mg daily for five days; at this point ingestion of the drug was discontinued. The reduction in dosage of diaphenylsulfone and the early cessation of treatment were decided upon because of the occurrence of methaemoglobinaemia exceeding 20% in this volunteer. Twenty-one-day courses of medication

were completed during studies with all other volunteers treated with diaphenylsulfone.

All data concerning the volunteers in these studies are given in the accompanying table.

Laboratory studies

Studies performed with all volunteers included: levels of the haematocrit (microhaematocrit method); levels of haemoglobin (cyanmethaemoglobin method) and methaemoglobin (Evelyn & Malloy, 1938); reticulocyte counts (brilliant cresyl-blue stain); erythrocyte, leukocyte, and differential leukocyte counts; platelet counts (direct method); levels of serum L-aspartate:2-oxoglutarate aminotransferase (serum glutamic oxalacetic transaminase; SGOT) and of blood urea nitrogen (BUN); levels of diaphenylsulfone in the blood; levels of reduced glutathione (GSH) in red cells; and studies of the survival and sequestration of ^{51}Cr -labelled red cells. Haematocrit and haemoglobin levels, reticulocyte counts, and levels of GSH in red cells were determined daily; the other studies were performed at less frequent intervals. Measurements of diaphenylsulfone in the urine, examinations of blood for the presence of Heinz bodies, determinations of levels of oxidized glutathione (GSSG) in red cells (Flanagan et al., 1958), and assays of glutathione reductase (Long & Carson, 1961), of G-6-PD, and of pyruvate kinase (Powell & DeGowin, 1965) in haemolysates were performed serially during studies with certain subjects.

Levels of diaphenylsulfone. Concentrations of diaphenylsulfone in samples of whole blood and in aliquots of 24-hour samples of urine were measured with a modification (Molesworth et al., 1949) of the Bratton & Marshall technique (1939). Mean optical density readings recorded during the baseline period were subtracted from values observed during treatment to correct for the small amounts of diazotizable substances normally present in blood or urine (Annino, 1964).

Levels of GSH. Levels of GSH in erythrocytes were determined with the nitroprusside method of Grunert & Phillips (1951); the precautions noted by Flanagan and co-workers (1958) were observed. During some of the studies with normal and G-6-PD-deficient volunteers treated with diaphenylsulfone, two methods were used simultaneously to measure levels of erythrocytic GSH; the second method employed was that described by Beutler et al. (1963). The findings observed with the two methods were

in close agreement. Both methods for measuring GSH were employed during additional studies carried out to determine whether or not diaphenylsulfone altered measurements of GSH; the levels of GSH detected in samples of whole blood, plasma, or saline to which diaphenylsulfone (2 mg per 100 ml) had been added were the same, both before and after incubation of the samples at 37°C, as those of corresponding control samples containing no added diaphenylsulfone. Similar findings have been reported by Bockris & Smith (1962). Since the maximum blood levels of diaphenylsulfone observed during the studies with volunteers were approximately 1 mg per 100 ml (see table), it appeared unlikely that diaphenylsulfone interfered with the determinations of levels of erythrocytic GSH.

Erythrocyte survival and sequestration studies. Studies of the survival of autologous ⁵¹Cr-labelled erythrocytes were performed as described by Tarlov & Kellermeyer (1961). The concentration of chromium never exceeded 0.08 µg per ml of blood during labelling of erythrocytes. The extent of haemolysis of labelled erythrocytes during ingestion of diaphenylsulfone was calculated by the method of Dern and co-workers (1955).

Two days after the administration of ⁵¹Cr-labelled erythrocytes, a scintillation probe was placed over the body surface above the spleen, liver, and heart. These sites were marked. Counting was performed for five minutes daily over each area initially and for 10 minutes daily when counts declined to less than 10 000 per minute; ratios (spleen: precordium and liver: precordium) of counts per minute were calculated. Mean ratios during the baseline period were subtracted from peak ratios observed subsequently to calculate over-all changes (in spleen: precordium or liver: precordium ratios) indicative of splenic or hepatic sequestration.

Other observations

The temperature, pulse, blood pressure, and body-weight of all volunteers were recorded daily; the only notable change observed in these values was slight fever in one G-6-PD-deficient subject (Volunteer 15) receiving 100 mg of diaphenylsulfone daily who developed a β-haemolytic streptococcal upper respiratory tract infection during the first week of ingestion of diaphenylsulfone. Symptoms and leukocytosis in Volunteer 15 subsided promptly after initiation of treatment with penicillin, and administration of the 21-day course of diaphenylsulfone to

this volunteer was continued and completed without interruption. Cyanosis, without other associated symptoms or signs, was usually evident in volunteers who developed methaemoglobinaemia exceeding 5%; other cutaneous changes were not observed. Some volunteers receiving 200 mg or 300 mg of diaphenylsulfone daily noted transient headaches that were not sufficiently bothersome to warrant discontinuation of the drug; although the findings suggested a causal relationship between the administration of diaphenylsulfone and the occurrence of headaches (Allday & Barnes, 1961) in some of these volunteers, the evidence was inconclusive because headaches were noted also by some of the control subjects not treated with diaphenylsulfone. Except for the transient leukocytosis noted in Volunteer 15, no remarkable changes in leukocyte counts or in differential leukocyte counts were detected. Platelet counts and levels of SGOT and of BUN remained normal. Thus, except during studies with Volunteer 15, evidence of possible complicating factors—including intercurrent infections, derangements involving platelets or leukocytes, or alterations of hepatic or renal function—was not observed.

RESULTS

Levels of diaphenylsulfone in the blood

Diaphenylsulfone was detected in the blood of all volunteers treated with this drug (see table).¹ The blood levels of diaphenylsulfone increased gradually during the first week of ingestion of this drug, remained relatively stable thereafter as long as administration of the same daily dose was continued, and then diminished relatively rapidly, to non-detectable levels, within two to four days after termination of treatment. In general, the mean diaphenylsulfone levels in the blood during the second week of ingestion (see table) were higher in volunteers receiving higher daily doses; however, there was considerable variation in the mean diaphenylsulfone levels in the blood of different individuals receiving the same daily dose, with overlapping of values in individuals receiving different daily doses. As shown in the table, the mean blood levels observed in G-6-PD-deficient subjects

¹ Diaphenylsulfone (or its metabolites) was detected in the urine of all the treated subjects from whom urine samples were obtained for study (Volunteers 1, 2, 3, 4, 6, 7, 9, 10, 16, and 17). The mean amount of diazotizable substances detected in the urine was equivalent to 63% of the daily dose of diaphenylsulfone.

DATA CONCERNING VOLUNTEERS PARTICIPATING IN STUDIES OF THE HAEMOLYTIC EFFECTS OF DIAPHENYLSULFONE

Volunteer	Race ^a	Age (years)	Weight (kg)	Activity of G-6-PD ^b	Mean level of erythrocytic GSH during the baseline period (mg/100 ml)	Daily dose of DDS (mg)	Mean level of DDS in the blood ^c (mg/100 ml)	Levels of the haematocrit		Peak level of methaemoglobinemia (%) ^d	Peak reticulocyte count (%)
								Mean baseline level (%)	Lowest value observed (%)		
Normal subjects											
1	C	36	71.4	264	72.9	300	0.42	44.8	34.4	18	7.3
2	C	26	63.6	277	66.9	300	0.59	48.0	37.2	5	9.4
3	N	24	82.3	321	80.1	300	0.95	46.2	37.0	22	5.3
4	N	33	75.4	291	66.8	300	0.98	44.6	39.8	9	6.2
5	N	27	82.7	316	55.3	200	0.60	49.2	45.5	14	5.0
6	C	26	80.9	273	72.6	200	0.24	47.8	42.5	2	3.2
7	N	28	78.6	229	64.6	200	0.42	45.0	39.2	7	4.6
8	N	23	73.2	263	82.9	100	0.13	43.5	36.9	6	3.1
9	N	36	87.7	282	64.9	100	0.15	46.3	42.2	3	2.8
10	N	24	80.9	236	69.7	50	0.04	46.1	42.0	1	3.8
11	C	33	71.8	310	75.4	none	0.00	45.4	41.0	<1	2.1
12	N	34	92.7	282	75.8	none	0.00	46.0	41.8	<1	2.6
13	N	32	68.2	268	66.9	none	0.00	46.0	41.5	<1	1.9
G-6-PD-deficient subjects											
14	N	27	80.0	23	51.9	200	0.45	45.5	34.5	9	9.2
15	N	25	78.2	23	47.4	100	0.10	45.6	33.5	6	13.1
16	N	25	74.1	17	47.0	100	0.19	50.2	44.0	2	7.0
17	N	38	81.8	20	48.4	50	0.07	45.5	36.5 ^e	2	6.1
18	N	35	71.4	15	50.9	25	0.04	47.6	44.5	<1	4.5
19	N	32	91.4	19	51.9	none	0.00	48.7	44.0	1	4.9

^a C = Caucasian; N = Negro.^b Activity of G-6-PD in haemolysates is expressed as μ mol of reduced triphosphopyridine nucleotide formed per g of haemoglobin per hour at 25°C.^c Values represent mean blood levels of diaphenylsulfone observed during the second week of ingestion of the drug.^d Methaemoglobinemia is expressed as percentage of total haemoglobin.^e A haematocrit level of 36.5 was reported on only one day during studies with this volunteer; on the preceding and subsequent days, the haematocrit levels in this volunteer were 39-40.

reated with diaphenylsulfone were within the range of values detected in normal subjects who received corresponding daily doses of the drug.

Haemolysis and levels of GSH in erythrocytes

The changes in levels of the haematocrit are summarized in the ninth and tenth columns of the table; the results of erythrocyte survival and sequestration studies are shown in Fig. 1 and 2; and the levels of GSH in erythrocytes are depicted in Fig. 3. The changes in haemoglobin levels and in erythrocyte counts paralleled changes in levels of the haematocrit. The findings made during studies with normal subjects differed strikingly from those detected during studies with G-6-PD-deficient subjects.

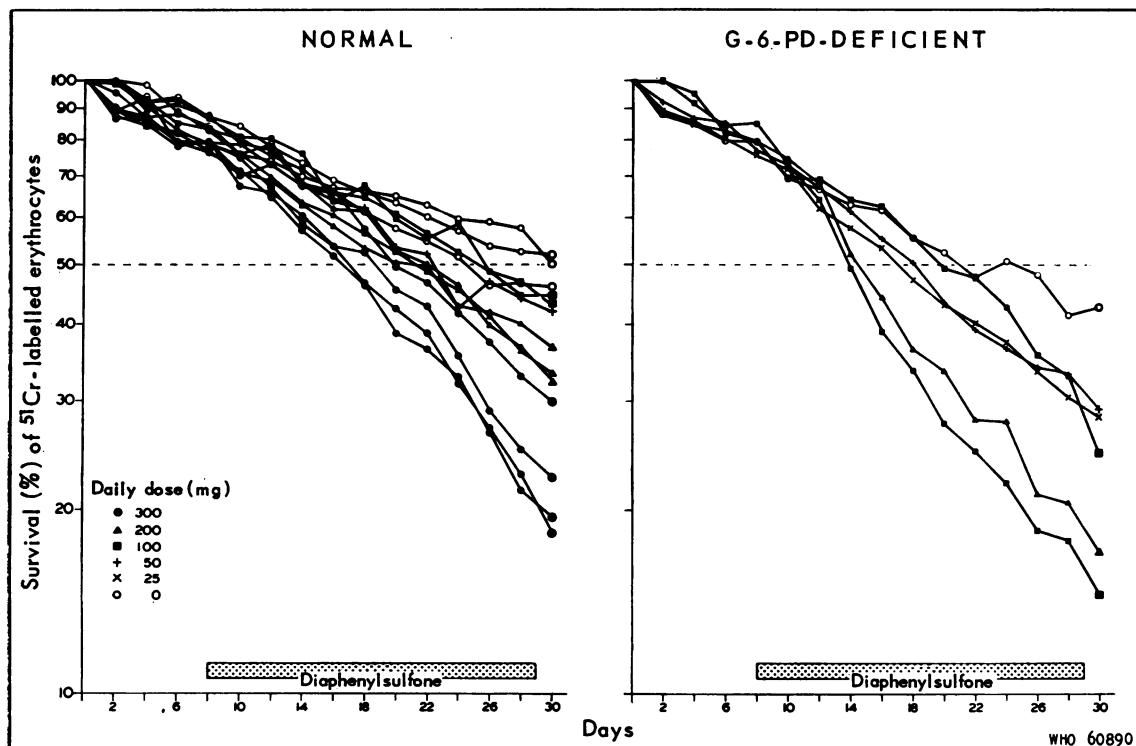
Studies with normal subjects. Haematocrit levels decreased by approximately 20%-25% (see table) and the survival of ^{51}Cr -labelled erythrocytes was shortened substantially (Fig. 1) in three of four normal subjects (Volunteers 1, 2, and 3) treated with 300 mg of diaphenylsulfone daily. In these three volunteers, the haematocrit levels decreased gradually during administration of diaphenylsulfone and then slowly returned to normal, in association with reticulocytosis, over several weeks after completion of treatment. The slopes of survival curves of erythrocytes of these three volunteers increased slightly during the first two weeks after initiation of treatment and then increased abruptly, suggesting accentuated haemolysis, during the third week (Fig. 1). Levels of GSH in erythrocytes of Volunteers 1, 2, and 3 increased during the first two weeks after initiation of treatment, decreased to or below the baseline levels during the third week, and then diminished to values well below the baseline levels the next week.¹ In all three subjects, diminutions in levels of erythrocytic GSH observed during the third week after initiation of treatment coincided temporally with abrupt increases in the slopes of erythrocyte survival curves. Fig. 4 shows the results of

studies with Volunteer 1 compared with findings observed with one of the normal control subjects.

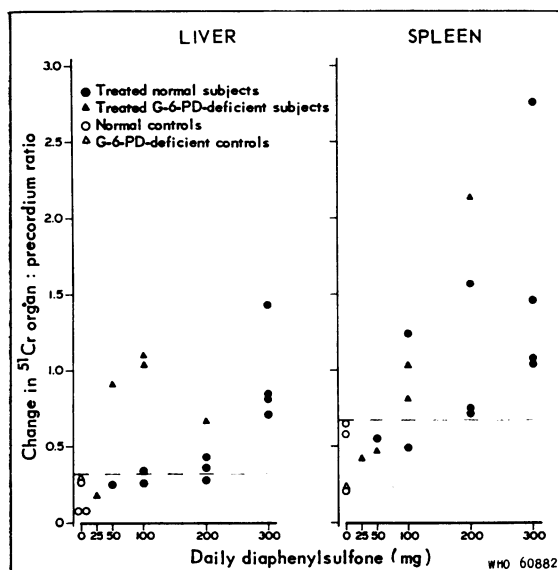
In the fourth normal subject who received 300 mg of diaphenylsulfone daily (Volunteer 4) and in the three normal subjects who received 200 mg of diaphenylsulfone daily (Volunteers 5, 6, and 7), levels of erythrocytic GSH increased slightly during the first week of treatment, remained elevated *throughout* the second and third weeks, and returned to or below baseline levels the next week (Fig. 3). Erythrocyte survival and sequestration studies (Fig. 1 and 2) yielded evidence of haemolysis in Volunteers 4, 5, 6, and 7, but the shortening of erythrocyte survival in these four volunteers was not as marked as in Volunteers 1, 2, and 3 (Fig. 1). In the normal subjects treated with 50 mg or 100 mg of diaphenylsulfone daily (Volunteers 8, 9, and 10), slight or equivocal increases in the levels of erythrocytic GSH occurred during administration of the drug (Fig. 3) and erythrocyte survival and sequestration studies yielded no or slight evidence of haemolysis (Fig. 1 and 2). Decreases in levels of the haematocrit in Volunteers 4-10 (see table) either were similar to or only slightly exceeded the small diminutions in the levels of the haematocrit, attributable to withdrawal of blood, that were observed in the normal control subjects (Volunteers 11, 12, and 13). Fig. 5 shows the results of studies with one of the normal subjects who received 100 mg of diaphenylsulfone daily compared with findings during studies with one of the normal control subjects.

Studies with G-6-PD-deficient subjects. Levels of GSH in erythrocytes of G-6-PD-deficient subjects treated with diaphenylsulfone decreased during the first week of treatment and then returned to or near baseline levels (Fig. 3), in sharp contrast to the sequence of changes in levels of erythrocytic GSH observed in normal subjects treated with diaphenylsulfone. Shortening of erythrocytic survival was more marked in G-6-PD-deficient subjects than in normal subjects receiving the same daily dose of diaphenylsulfone (Fig. 1). In the G-6-PD-deficient subject treated with 200 mg of diaphenylsulfone daily (Volunteer 14) and in the G-6-PD-deficient subject treated with 100 mg daily who developed an intercurrent streptococcal infection (Volunteer 15), shortening of erythrocytic survival (Fig. 1) and decreases in levels of erythrocytic GSH during the first week of treatment (Fig. 3) were considerably more pronounced than in the G-6-PD-deficient subject receiving 100 mg daily who did not develop an

¹ During a subsequent study carried out with an additional normal volunteer (not listed in the table) who received 300 mg of diaphenylsulfone daily for 14 days, the levels of erythrocytic GSH increased during ingestion of diaphenylsulfone and then decreased to values well below the baseline (pre-drug) levels after completion of treatment; long-term follow-up studies revealed that, subsequently, the levels of GSH in erythrocytes of this normal subject very gradually returned to or near baseline values over approximately six weeks after completion of administration of diaphenylsulfone. A long-lasting effect upon levels of GSH in erythrocytes of normal persons has been observed previously during studies involving administration of acetylphenylhydrazine to normal individuals (Flanagan et al., 1958).

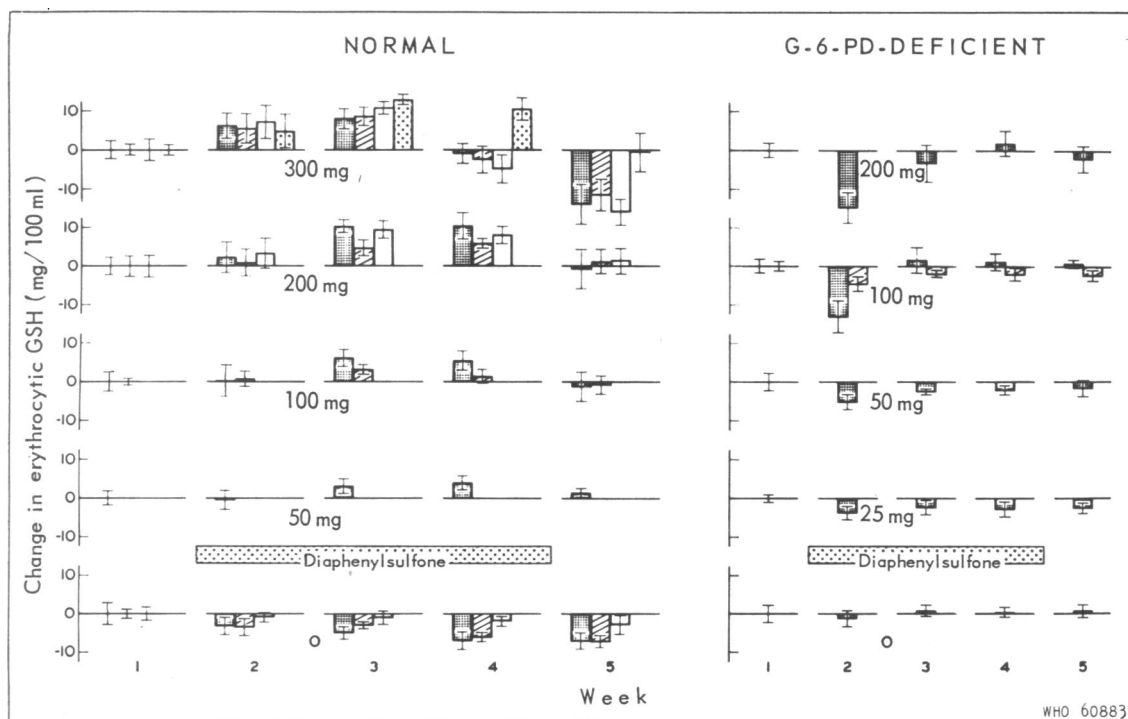
FIG. 1. DOSAGE OF DIAPHENYLSULFONE AND ERYTHROCYTE SURVIVAL IN ALL 19 VOLUNTEERS ^a

^a The three normal volunteers in whom shortening of erythrocytic survival was most marked were Volunteers 1, 2 and 3, and the two G-6-PD-deficient volunteers in whom shortening of erythrocytic survival was most marked were Volunteers 14 and 15 (see table). Volunteers 2 and 3, as indicated in the text, did not receive full 21-day courses of diaphenylsulfone.

FIG. 2. BODY SURFACE RADIOACTIVITY ^a

^a This figure shows over-all changes in ratios (liver: precordium and spleen: precordium) of body surface radioactivity calculated as indicated in the text. Evidence of both hepatic and splenic sequestration of labelled erythrocytes was observed in normal and in G-6-PD-deficient subjects treated with diaphenylsulfone.

FIG. 3
EFFECT OF DIAPHENYLSULFONE ON ERYTHROCYTE REDUCED GLUTATHIONE^a



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^a This figure depicts mean changes in levels of reduced glutathione in erythrocytes of each subject during each week of the investigations. Brackets enclose one standard deviation above and below the means. Mean levels of erythrocytic GSH observed during the first week (baseline period) of each study are noted in the table; mean levels during the baseline period were subtracted from mean levels observed during each subsequent week to yield the changes shown in this figure. Data for each subject each week are presented in the same order as in the table. For example, at the top in the left-hand section of the figure (normal subjects), data for Volunteers 1, 2, 3, and 4 (Table 1) are presented in that order for each week above the designation indicating a daily dose of 300 mg of diaphenylsulfone; and data for Volunteers 5, 6, and 7 are presented in that order for each week above the designation indicating a daily dose of 200 mg. The normal subject receiving 300 mg daily in whom levels of erythrocytic GSH remained elevated during the third week of treatment was Volunteer 4, and the G-6-PD-deficient subject receiving 100 mg daily in whom levels of erythrocytic GSH decreased markedly during the first week of treatment was Volunteer 15.

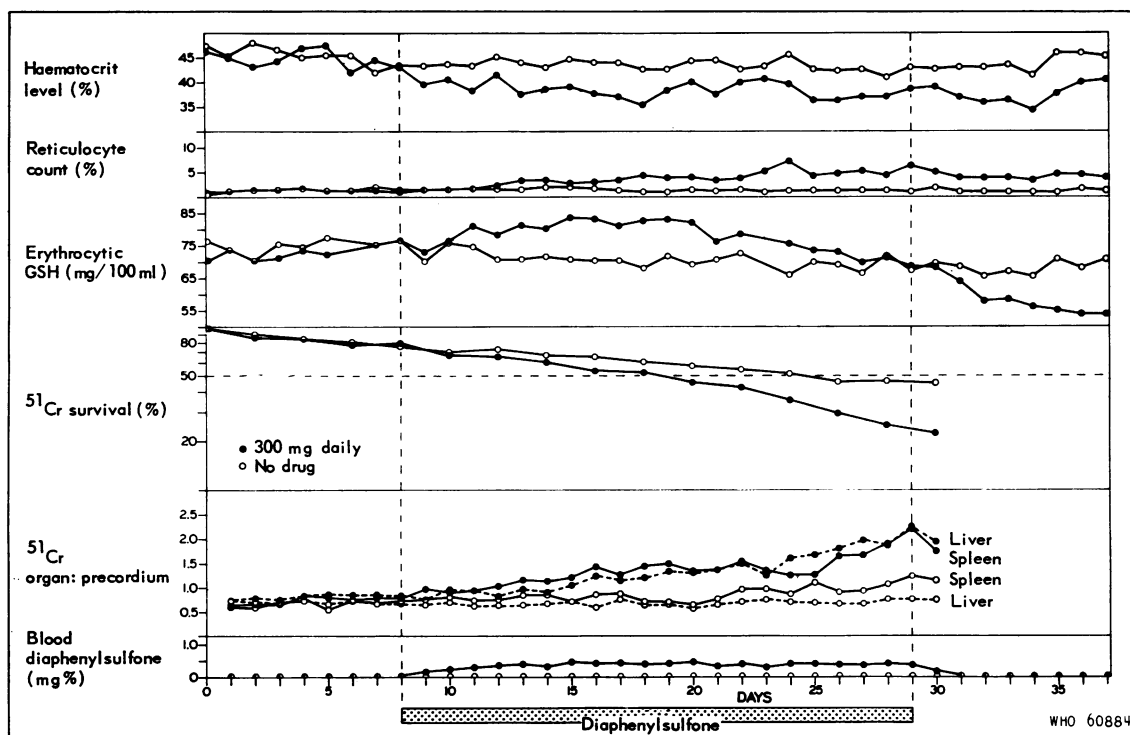
intercurrent infection (Volunteer 16) or in the G-6-PD-deficient subjects who received 50 mg or 25 mg of diaphenylsulfone daily (Volunteers 17 and 18).

Changes in levels of the haematocrit in Volunteers 16, 17, and 18 were similar to or only slightly exceeded the small decrease noted in the G-6-PD-deficient control subject (Volunteer 19) who received no diaphenylsulfone, whereas levels of the haematocrit in Volunteers 14 and 15 decreased by approximately 20%-25% (see table). The levels of GSH in erythrocytes of Volunteers 14 and 15 began to decrease one to two days after initiation of treatment and reached their lowest values during the third to fourth days of treatment. Levels of the haematocrit in Volunteers 14 and 15 began to decline on the

fourth to fifth days of treatment, concomitant with a sharp increase in the slopes of erythrocytic survival curves (Fig. 1), and they reached their lowest values during the second week of treatment and then gradually increased in association with reticulocytosis. Thus, in both subjects, levels of erythrocytic GSH decreased prior to the onset of acute haemolysis.

Fig. 6 shows the results of studies with Volunteer 14, the G-6-PD-deficient subject who received 200 mg of diaphenylsulfone daily, compared with findings observed during studies with one of the normal volunteers (Volunteer 7) who received an identical daily dose and in whom blood diaphenylsulfone levels were very similar to those detected in Volunteer 14.

FIG. 4
EFFECT OF 300 mg DIAPHENYLSULFONE DAILY ON NORMAL ERYTHROCYTES ^a



^a Data for Volunteer 1 (300 mg daily) compared with Volunteer 11 (untreated control).

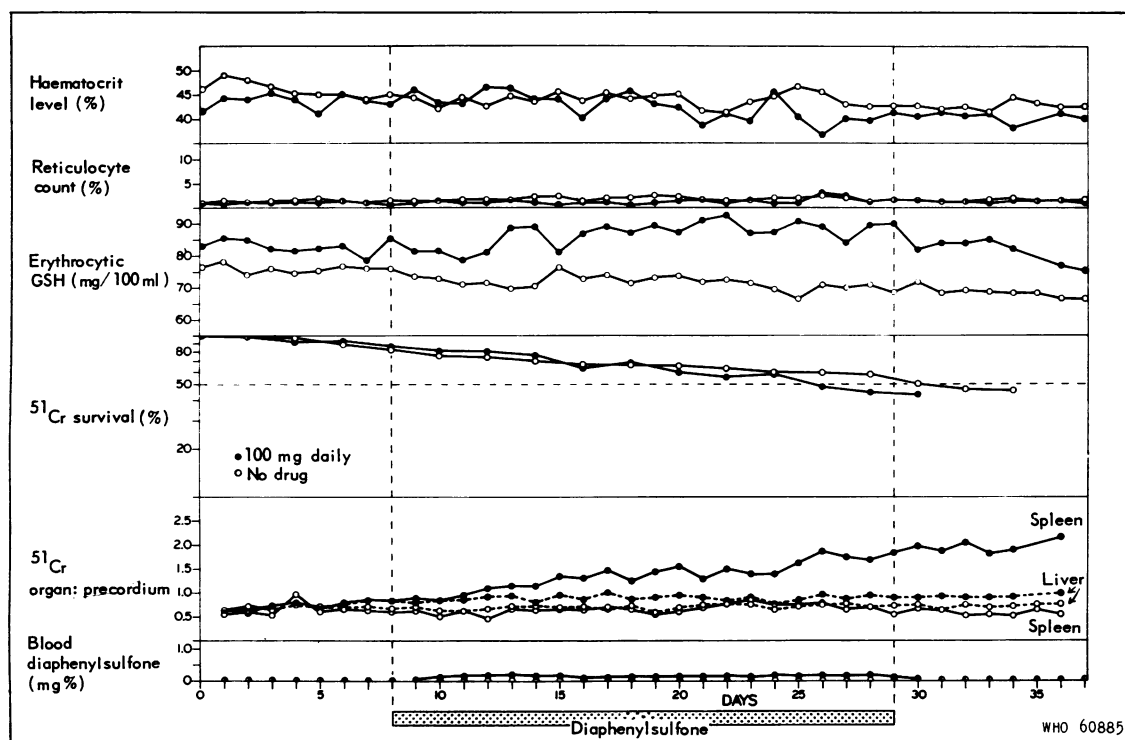
Other studies

Methaemoglobinaemia exceeding 2% was detected in 10 of 12 volunteers treated with 100 mg or more of diaphenylsulfone daily, but not in the three subjects who received 25 mg or 50 mg daily. In subjects treated with 100 mg or more daily, methaemoglobinaemia usually increased slowly during the first week of treatment in parallel with the gradually increasing blood levels of diaphenylsulfone. Peak levels of methaemoglobinaemia occurred during the second week of treatment, and there was a return to normal values within three to four days after termination of treatment. Peak levels of methaemoglobinaemia varied considerably. The findings did not suggest a marked difference in levels of methaemoglobinaemia in G-6-PD-deficient compared with normal subjects treated with the same daily dose of diaphenylsulfone. Examinations for Heinz bodies were performed serially during studies with 12 volunteers treated with diaphenyl-

sulfone; in agreement with findings of other workers (Smith & Alexander, 1959; Gisslén & Hersle, 1964), Heinz bodies were detected in the erythrocytes of nine of these volunteers.

Serial determinations of the content of GSSG in erythrocytes and measurements of the activities of glutathione reductase and G-6-PD in haemolysates, performed during studies with seven volunteers who received diaphenylsulfone and with three control subjects (Volunteers 4, 6, 7, 9, 10, 12, 13, 16, 18, and 19), disclosed no significant changes in levels of erythrocytic GSSG and no evidence of inhibition of glutathione reductase or of G-6-PD during the period of study. In association with reticulocytosis, very slight or equivocal increases in glutathione reductase activity were observed in two of the normal volunteers treated with diaphenylsulfone (Volunteers 4 and 9) and increases in G-6-PD activity were observed in three of the normal subjects (Volunteers 4, 6, and 7) and in two of the G-6-PD-deficient subjects (Volunteers 16 and 18) so treated. Serial

FIG. 5
EFFECT OF 100 mg DIAPHENYLSULFONE DAILY ON NORMAL ERYTHROCYTES^a



^a Data for Volunteer 8 (100 mg daily) compared with Volunteer 12 (untreated control).

determinations of the activity of pyruvate kinase in haemolysates, performed during studies with three volunteers treated with diaphenylsulfone and with one control subject (Volunteers 4, 9, 13, and 18), disclosed no significant change in activity prior to the onset of reticulocytosis and a marked increase in activity in association with reticulocytosis in the three subjects studied who received diaphenylsulfone. Fig. 7 shows the results of studies on the activity of pyruvate kinase in haemolysates of erythrocytes of one of the normal treated subjects (Volunteer 4), who received 300 mg of diaphenylsulfone daily, compared with results observed during studies with a normal control subject (Volunteer 13).

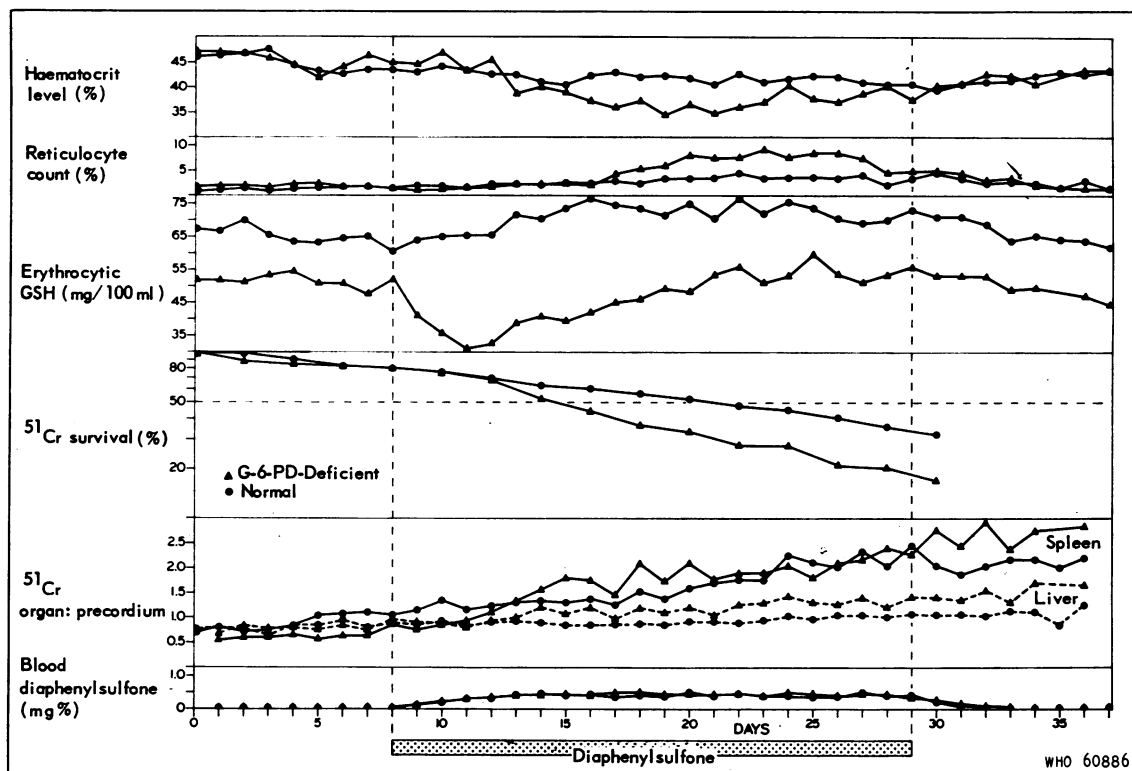
The haemolytic effect of diaphenylsulfone compared with the haemolytic effect of primaquine

Calculations of the percentage of erythrocytes lysed during administration of diaphenylsulfone disclosed a reasonably linear relationship between the

extent of haemolysis and the dose of diaphenylsulfone, expressed in mg per kg body-weight per day, both in normal subjects and in G-6-PD-deficient subjects (Fig. 8); Volunteer 15, the G-6-PD-deficient subject who had an intercurrent streptococcal infection, was a notable exception. Similar calculations, based upon investigations conducted previously at this laboratory in which erythrocytic survival studies were performed during administration of primaquine daily for 14-18 days to normal and to G-6-PD-deficient subjects, indicated that the haemolytic effect of diaphenylsulfone, although more pronounced than that of primaquine in normal men, is less pronounced than that of primaquine in Negro men with G-6-PD deficiency (Fig. 8).

The volunteers participating in the studies of primaquine-induced haemolysis depicted in Fig. 8 were individuals different from those participating in the studies presented in this report, with one exception. One G-6-PD-deficient volunteer who

FIG. 6
EFFECT OF 200 mg DIAPHENYLSULFONE DAILY ON NORMAL AND G-6-PD-DEFICIENT ERYTHROCYTES^a



^a Data for G-6-PD-deficient Volunteer 14 (200 mg daily) compared with normal Volunteer 7 (200 mg daily).

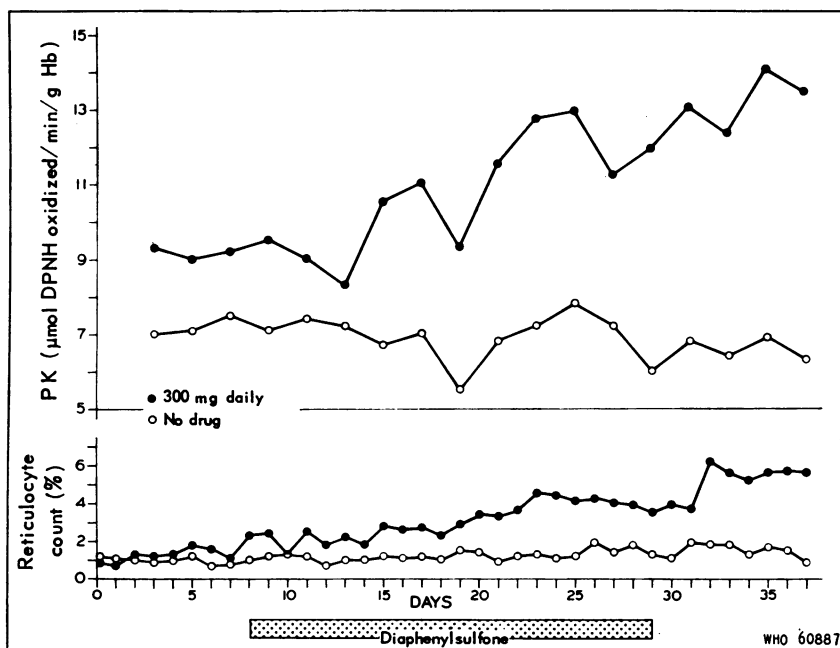
received 100 mg of diaphenylsulfone daily for 21 days during the present studies (Volunteer 16) had participated in studies on the haemolytic effects of primaquine one year previously. At that time he had received 30 mg of primaquine base daily for 18 days. Fig. 9 shows the results of both studies with Volunteer 16. In this G-6-PD-deficient individual, the haemolytic effect resulting from administration of 100 mg of diaphenylsulfone daily for 21 days was less marked than that resulting from administration of 30 mg of primaquine base daily for 18 days.

DISCUSSION

The studies presented in this report demonstrate clearly that G-6-PD-deficient American Negro men are more susceptible to diaphenylsulfone-induced haemolysis than are persons without this deficiency.

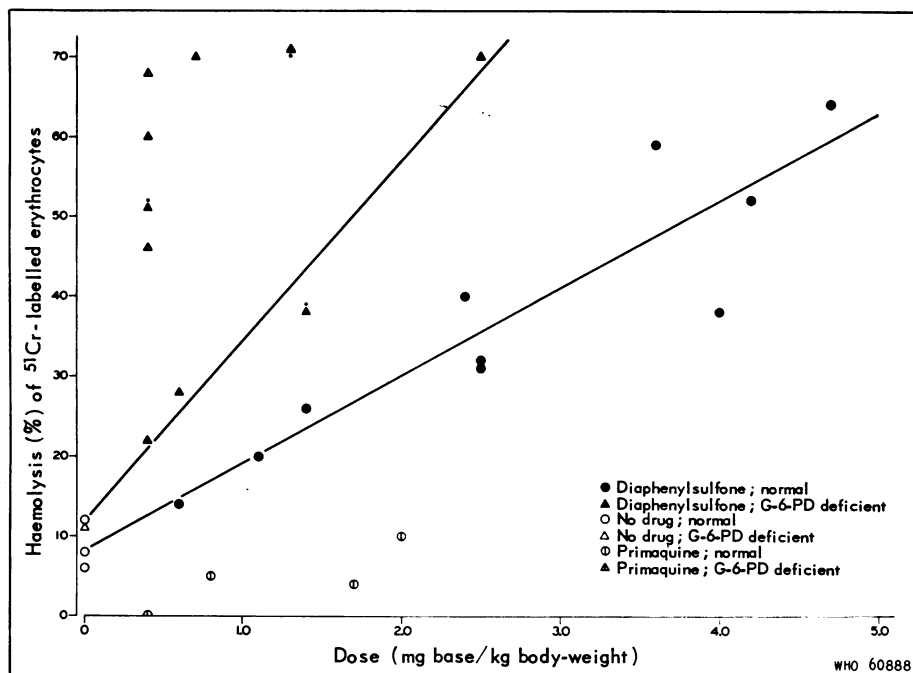
Except for studies with one G-6-PD-deficient man who had an intercurrent streptococcal infection, the findings indicate that, both in normal individuals and in G-6-PD-deficient Negro men, there is a direct relationship between the dose of diaphenylsulfone and the extent of haemolysis that occurs during relatively short-term daily administration of the drug. The relationships between the dose and the extent of haemolysis are apparent only when normal and G-6-PD-deficient individuals are considered in separate groups. If the results of the studies with normal and G-6-PD-deficient subjects were considered together, without knowledge of the predisposition to drug-induced haemolysis associated with G-6-PD-deficiency, haemolysis or haemolytic anaemia induced by administration of diaphenylsulfone might appear to be almost capricious in occurrence or in degree.

FIG. 7. EFFECT OF 300 mg DIAPHENYLSULFONE DAILY ON ERYTHROCYTE PYRUVATE KINASE ACTIVITY AND RETICULOCYTE COUNTS^a



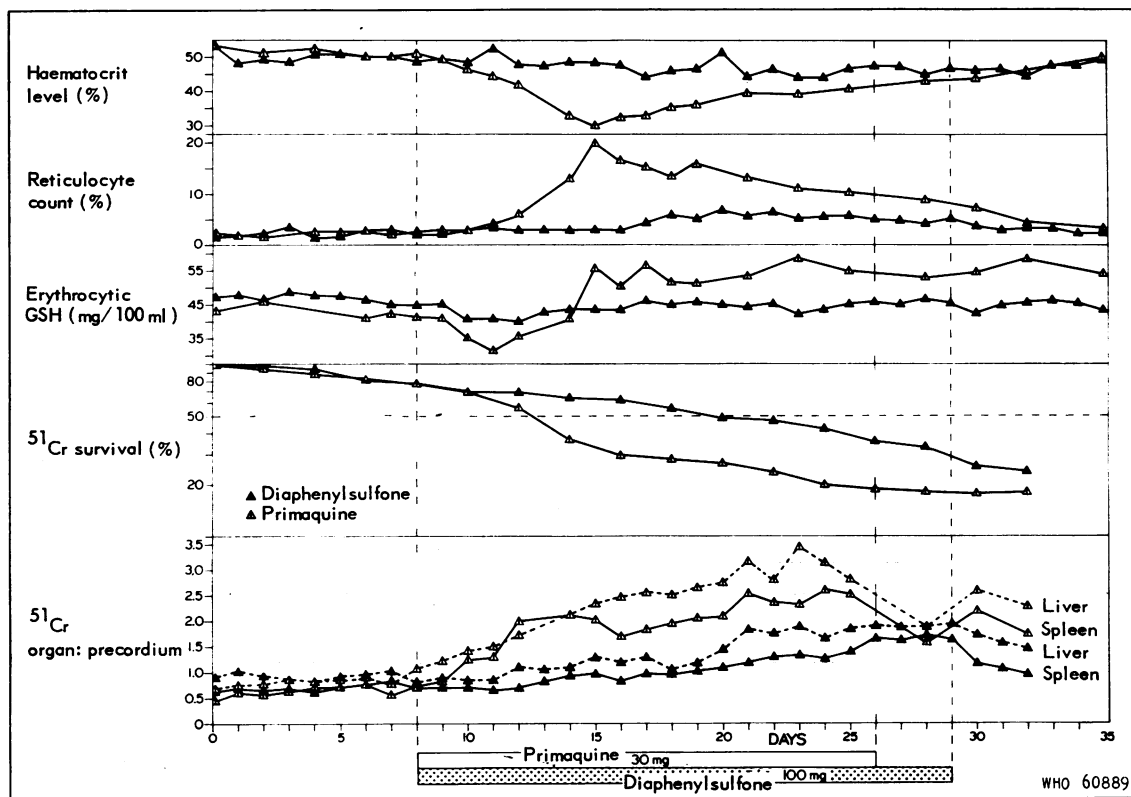
^a Data for Volunteer 4 (300 mg daily) compared with Volunteer 13 (untreated control).

FIG. 8. RELATIONSHIP BETWEEN DOSAGE OF DRUG AND HAEMOLYSIS^a



^a The triangle with a dot underneath indicates results of studies with the G-6-PD-deficient subject who developed an inter-current streptococcal infection (Volunteer 15). The lines reflect calculations by the method of least squares excluding data with Volunteer 15. Triangles with dots above indicate results of studies with Volunteer 16, who received diaphenylsulfone during the current studies and who had received primaquine during previous studies.

FIG. 9
EFFECT OF DIAPHENYLSULFONE AND PRIMAQUINE ON G-6-PD-DEFICIENT ERYTHROCYTES^a



^a Results of two different studies with Volunteer 16 (one involving administration of diaphenylsulfone and the other involving administration of primaquine) carried out at different times superimposed for purposes of comparison (see text).

The results of the erythrocyte survival studies carried out with G-6-PD-deficient Negro men treated with diaphenylsulfone are consistent with findings reported previously by Dern, Beutler & Alving (1955). They observed that administration of aldesulfone¹ (sulfoxone; a disubstituted derivative of diaphenylsulfone) caused haemolysis of transfused "primaquine-sensitive" erythrocytes in normal recipients. The results of the erythrocyte survival studies in normal men treated with diaphenylsulfone are consistent with observations reported by Pengally (1963), who studied individuals not G-6-PD-deficient and detected shortened survival of ⁵¹Cr-labelled erythrocytes, without frank anaemia, in one healthy person and in four patients with dermatitis herpetiformis treated with 100 mg to 200 mg of diaphenylsulfone

daily. Pengally noted that some workers have stated that haemolytic anaemia occurs to a varying degree in practically every patient taking sulfone drugs, whereas others have stated that haemolytic anaemia due to diaphenylsulfone is rare, and he pointed out that these statements would be compatible if the former workers had employed the term "haemolysis" rather than "haemolytic anaemia". Our findings underscore this point and emphasize also that other important factors include the dose of diaphenylsulfone and the presence or absence of G-6-PD deficiency.

Intercurrent infections may increase the severity of drug-induced haemolysis in G-6-PD-deficient individuals (Tarlov et al., 1962). The intercurrent β -haemolytic streptococcal infection in one of the G-6-PD-deficient volunteers treated with 100 mg of diaphenylsulfone daily (Volunteer 15) may have

¹ Proposed International Non-Proprietary Name.

enhanced the severity of diaphenylsulfone-induced haemolysis in this subject. Haemolysis in Volunteer 15 considerably exceeded that in the other G-6-PD-deficient volunteer treated with 100 mg of diaphenylsulfone daily and was similar, in severity, to that occurring in the G-6-PD-deficient subject (Volunteer 14) who received 200 mg of diaphenylsulfone daily.

The levels of GSH in erythrocytes of Volunteers 14 and 15 decreased markedly just before and early during the acute haemolytic episode that resulted from administration of diaphenylsulfone. They then increased to or above baseline levels as administration of diaphenylsulfone to these two G-6-PD-deficient volunteers was continued. The changes in levels of GSH detected in erythrocytes of Volunteers 14 and 15 are very similar to those known to occur (Flanagan et al., 1958) during haemolysis induced by administration of 30 mg of primaquine base daily to G-6-PD-deficient Negro men.

In sharp contrast to the changes in the erythrocytic GSH levels detected in G-6-PD-deficient subjects treated with diaphenylsulfone, those levels in normal subjects increased during administration of the drug. During studies with normal subjects treated with 200 mg or 300 mg of diaphenylsulfone daily, slight but definite haemolysis occurred in the face of slightly increased levels of GSH during the first two weeks of ingestion of diaphenylsulfone. In three normal subjects receiving 300 mg daily (Volunteers 1, 2, and 3), an acceleration of haemolysis occurred concomitant with decreases in levels of erythrocytic GSH during the third week after initiation of treatment; the latter findings are somewhat comparable to changes detected initially during haemolysis induced by administration of diaphenylsulfone to G-6-PD-deficient Volunteers 14 and 15.

Increases in the levels of GSH in erythrocytes of normal subjects treated with diaphenylsulfone consistently occurred before the onset of reticulocytosis in these subjects; the initial increases in levels of erythrocytic GSH observed, therefore, cannot be explained on the basis of possibly increased levels of GSH in very young cells released into the circulation in response to haemolysis. Thus, the possibility exists that diaphenylsulfone caused increased formation of GSH in normal erythrocytes.

An unusually high level of GSH was detected in erythrocytes of a 2-year-old girl who accidentally ingested an unknown quantity of diaphenylsulfone (Brockris & Smith, 1962; Stanfield, 1963). The girl had been treated with methylene blue because of methaemoglobinemia. Brockris & Smith (1962)

observed an increase in levels of erythrocytic GSH in rabbits treated with methylene blue; they attributed the high level of GSH detected in erythrocytes of the 2-year-old girl to increased formation of GSH in response to the methylene blue she had received. Methylene blue is one of the many compounds capable of causing haemolysis in G-6-PD-deficient individuals (Brewer & Tarlov, 1961). During studies at our laboratory in recent years, increases in levels of erythrocytic GSH, like those detected by Brockris & Smith during their studies with rabbits, have not been observed during studies with normal or G-6-PD-deficient human beings treated with methylene blue.

Another possible explanation for the initial increases in levels of GSH in erythrocytes of normal subjects treated with diaphenylsulfone is that circulating erythrocytes having relatively low levels of GSH were preferentially destroyed, leaving those having relatively high GSH levels. Older circulating erythrocytes of G-6-PD-deficient American Negro men are preferentially destroyed during primaquine-induced haemolysis (Beutler et al., 1954). Desforges and co-workers (1959) have reported that aldesulfone (sulfoxone) caused haemolysis of older circulating erythrocytes in a patient with leprosy. Older circulating erythrocytes may be preferentially destroyed during haemolysis induced by administration of diaphenylsulfone to normal or to G-6-PD-deficient persons; our findings do not provide conclusive evidence concerning this question, but they are consistent with this possibility. Many biochemical changes occur in human erythrocytes as the cells age *in vivo* (Allison & Burn, 1955; Pranker, 1961; Brewer et al., 1964), including substantial decreases in the activities of several key erythrocytic enzymes such as G-6-PD and pyruvate kinase (Marks & Gross, 1959; Pranker, 1961; Powell & DeGowin, 1965); our findings of increased activities of G-6-PD and pyruvate kinase associated with reticulocytosis in subjects treated with diaphenylsulfone are consistent with previous observations indicating that the activities of these two enzymes are higher in young than in old erythrocytes. Although more information is needed (Spear & Sass, 1964), some investigations do suggest that the level of GSH in normal human erythrocytes does not decrease as the cells age *in vivo* (Pranker, 1958; Rigas & Koler, 1961).

A chronic nonspherocytic haemolytic anaemia has been observed in patients who have an inherited deficiency of erythrocytic GSH, and erythrocytes of these patients have been found to be susceptible

to primaquine-induced haemolysis (Prins et al., 1966). The data concerning patients who have an inherited deficiency of erythrocytic GSH and the results of studies of haemolysis induced by primaquine, diaphenylsulfone, and certain other drugs strongly suggest that, although GSH may not be absolutely essential for erythrocyte viability, GSH in the erythrocyte is important for survival of the cell both in the absence and in the presence of haemolytic agents. Our findings suggest that normal erythrocytes have a greater capacity to resist diaphenylsulfone-induced haemolysis than do G-6-PD-deficient erythrocytes, and that the greater capacity of normal erythrocytes to resist diaphenylsulfone-induced lysis may reflect the ability of the normal cells to increase formation of GSH. However, the capacity to resist diaphenylsulfone-induced lysis displayed by many normal erythrocytes that survive initially during administration of diaphenylsulfone may be overcome, thereby requiring additional compensatory formation and release of young erythrocytes by the bone marrow, if administration of a sufficiently high dose of diaphenylsulfone is continued.

Additional evidence is needed concerning diaphenylsulfone-induced haemolysis in persons receiving long-term treatment and in individuals who have different genetic variants of G-6-PD deficiency or who have inherited erythrocytic derangements other than G-6-PD deficiency that may also predispose to drug-induced haemolysis. Erythrocytic alterations in persons receiving long-term therapy with diaphenylsulfone may differ from those noted during relatively short-term treatment. G-6-PD deficiency, in general, appears to be more extensive and more severe, and susceptibility to drug-induced haemo-

lysis may be more marked, in affected Caucasians or in affected Chinese than in affected Negroes (Chan et al., 1965).

Alertness to the hypersusceptibility to diaphenylsulfone-induced haemolysis displayed by G-6-PD-deficient individuals is warranted during clinical use of diaphenylsulfone. The general trend in the use of diaphenylsulfone for treatment of leprosy, especially early during therapy, has been to employ lower or less frequent doses of the drug than those used formerly (Bushby, 1964); however, doses somewhat higher than those now often used initially for therapy of leprosy have at times been considered necessary when diaphenylsulfone has been employed in the treatment of dermatitis herpetiformis or of certain other chronic dermatological disorders (Morgan et al., 1955; Linn, 1962; Lorincz & Pearson, 1963). Clearly, the administration of doses such as 200 mg of diaphenylsulfone daily may cause severe haemolysis in individuals having fully expressed G-6-PD deficiency. Recent investigations have disclosed that the administration of 25 mg or 50 mg of diaphenylsulfone daily exerts a substantial suppressive effect on infections with certain strains of chloroquine-resistant *Plasmodium falciparum* from South-East Asia (DeGowin et al., 1966). The studies presented in this report, as well as long-term investigations that have been under way in our laboratory for more than six months, indicate that, both in normal persons and in healthy G-6-PD-deficient American Negro men, marked haemolysis does not attend daily administration of relatively low doses of diaphenylsulfone such as those found effective in suppression of infections with chloroquine-resistant *P. falciparum*.

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RÉSUMÉ

L'emploi croissant des sulfones comme antipaludiques dans les régions où *Plasmodium falciparum* a été trouvé résistant à la chloroquine justifie l'étude de l'hémolyse qu'elles provoquent. Cet effet secondaire indésirable présente de nombreux aspects mal connus malgré l'usage

intensif qui a été fait des sulfones dans le traitement de la lèpre.

Les auteurs ont procédé à une étude détaillée de l'action hémolytique de la diamino-4,4'-diphénylsulfone (DDS) administrée à des sujets normaux, ou présentant une

carence en glucose-6-phosphate déshydrogénase (G-6-PD). Quinze volontaires, dix normaux et cinq atteints de carence en G-6-PD, ont reçu par voie buccale, pendant 21 jours, des doses de DDS variant de 25 mg à 300 mg par jour. Quatre autres, trois sujets normaux et un sujet carencé en G-6-PD, ont servi de témoins. Tous étaient en excellente santé, comme l'a confirmé un examen médical approfondi.

Dans les deux groupes, sujets normaux et carencés en G-6-PD, on a constaté une relation directe entre la dose de DDS exprimée en mg par kg de poids corporel et par jour et l'importance de l'hémolyse provoquée. A doses égales par kg de poids corporel, la DDS se montre plus hémolytique que la primaquine chez les sujets normaux et moins hémolytique chez les sujets carencés en G-6-PD. Le taux de glutathion réduit présent dans les érythrocytes

varie également de façon différente: il augmente précocement au début de l'hémolyse provoquée par la DDS chez l'homme normal; chez les sujets carencés en G-6-PD, au contraire, il diminue de façon marquée immédiatement avant les épisodes hémolytiques aigus provoqués par l'administration de DDS et au début de ces épisodes.

Les auteurs soulignent que l'administration de doses atteignant 200 mg de DDS par jour peut provoquer des hémorragies graves, mais des études récentes ont montré que l'administration quotidienne de 25 à 50 mg de DDS a une action suppressive marquée sur l'infection due à certaines souches de *P. falciparum* résistantes à la chloroquine. D'après les résultats de leurs recherches, l'hémolyse n'apparaît à cette dose ni chez les sujets normaux ni chez les sujets présentant une carence en G-6-PD.

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